two 66 bp *hin*FI fragments from bacteriophage ϕX174 (Life Technologies, Inc.). Gel mobility shift experiments were performed as described (Hengen *et al.* (1997) *supra.*).--

Please replace the paragraph beginning at page 55, line 2, with the following:

--A single very long nucleic acid (SEQ ID NO:15) is synthesized having a sequence in the center that causes a hairpin loop to form rapidly (*see,* Figure 11). The entire DNA can then be dissolved in a buffer heated and cooled thereby forming double stranded DNA. This guarantees that the complementary strands are equimolar and there isn't any single-stranded DNA present in the mixture.--

Please insert the accompanying paper copy of the Sequence Listing, page numbers 1 to 5, at the end of the application.

REMARKS

Applicants request entry of this amendment in adherence with 37 C.F.R. §§1.821 to 1.825. This amendment is accompanied by a floppy disk containing the above named sequences, SEQ ID NOS:1-19, in computer readable form, and a paper copy of the sequence information which has been printed from the floppy disk.

The information contained in the computer readable disk was prepared through the use of the software program "PatentIn" and is identical to that of the paper copy. This amendment contains no new matter.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,

Jean M. Lockyer
Reg. No. 44,879

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
Tel: (415) 576-0200
Fax: (415) 576-0300
DMW

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Paragraph beginning at line 23 of page 12 has been amended as follows:

Figure 7 illustrates the self-similarity of Fis binding sites. The sequence logo for Fis (SEQ ID NO:4) (Schneider & Stephens (1990) *Nucl. Acids Res.*, 18: 6097-6100; Hengen *et al.* (1997) *Nucl. Acids Res.*, 25(24): 4994-5002) is shown three times. The upper and lower logos are shifted +11 and +7 bases to the right (respectively) relative to the middle logo. The cosine wave, with a wavelength of 10.6 bases, shows that the +11 relatively shifted Fis sites would be on the same face of the DNA, while the +7 relatively shifted Fis sites would be on opposite faces. Arrows are at positions where the logo is self-similar after a shift. Down arrows mean that the contacts by Fis to the bases would interfere because they would be on the same face of the DNA. Up arrows mean that the contacts could be simultaneous because they are on opposite faces.

Paragraph beginning at line 1 of page 13 has been amended as follows:

Figures 8a, 8b, and 8c illustrate the oligonucleotide design of overlapping and separated Fis binding sites. The predicted Fis sites are shown by walkers floating below each DNA sequence (Schneider (1997) *Nucl. Acids Res.*, 25: 4408-4415; Hengen *et al.* (1997) *supra.*). In a walker, the vertical box marks the zero base of the binding site. The box also shows the vertical scale, with the upper edge being at +2 bits and the lower edge being at -3 bits. The height of each letter is determined from the bit value in the Riw(b,l) matrix (Schneider (1997) *J. Theoret. Biol.*, 189(4): 427-441; Schneider (1997) *Nucl. Acids Res.*, 25: 4408-4415; Hengen *et al.* (1997) *supra.*). Negative weights are represented by drawing the letter upside-down and placing it below the zero bit level. Three DNAs were designed, each having two Fis sites spaced 11 (SEQ ID NO:2), 7 (SEQ ID NO:3) and 23 (SEQ ID NO:5) bases apart. Design details are given in Example 1, Materials and Methods. The total strength of a site is the sum of the information weights for each base. The 18.1 bit Fis sites (SEQ ID NOS: 6 and 7) are 3.4 standard deviations higher than the average Fis site in natural sequences (Hengen *et al.* (1997) *supra.*; Schneider (1997) *J. Theoret. Biol.*, 189(4):427-441). The 12.7 (SEQ ID NOS:8 and 9) and 15.0 (SEQ ID NOS:10 and 11) bit sites are 1.6 and 2.4 standard deviations above average (respectively).

Paragraph beginning at line 28 of page 13 has been amended as follows:

Figure 10 shows the positions of Fis and DnaA sites at the *Escherichia coli* origin of replication (*oriC*). Sequence data are from GenBank accession K01789. The horizontal dashes below the sequence (SEQ ID NO:12) represent regions protected by Fis. Locations of DnaA sites are from Messer *et al.* (1991) *Res. Microbiol,* 142: 119-125). The asymmetric DnaA individual information matrix was created from 27 experimentally demonstrated DnaA binding sites (data not shown). DNA synthesis start sites are indicated by the arrows at the bottom (Seufert & Messer (1987) *EMBO J.* 6: 2469-2472). The boxes mark two Fis sites separated by 11 bases (SEQ ID NOS:13 and 14). Fis sites with positive individual information are marked from -7 to +7 but evaluated from -10 to +10 according to the matrix. DnaA site directionality is indicated by letters turned sideways in the direction that DnaA binds (Schneider (1997) *Nucl. Acids Res.*, 25: 4408-4415).

Paragraph beginning at line 11 of page 54 has been amended as follows:

Synthetic DNAs containing strong Fis sites separated by 11 and 7 base pairs were designed by selecting from the most frequent bases at each position in the Fis sequence logo (Hengen *et* al. (1997) *supra.*). These were then merged with the same sequence shifted by 11 or 7 base pairs by comparing the $R_{iw}(b,l)$ values for various choices. (Note: the consensus sequence of the early model we used was TTTG(G/C)TCA AAATTTGA(G/C)C AAA (SEQ ID NO:1) ~~(SEQ ID NO: 4)~~ which differs from that of the logo.) Five extra bases were added to the ends based on the natural sequences around the *hin* proximal and medial sites for the overlap 11 oligo, and the sequences around *cin* external and proximal sites were used for the overlap 7 oligo (Hengen *et al.* (1997) *supra.*). The DNAs were made self complementary (Fig. 8a, 8b). Sites separated by 23 bases were created starting with the 11 base separated DNA and duplicating the central overlap region. A *Bam*HI site was also inserted and the DNA was flanked by *Eco*RI sites (Fig. 10c). Oligonucleotides were synthesized with biotin on the 5' end and gel purified (Oligos Etc., Wilsonville, OR, USA). To ensure thorough annealing, they were heated to 90°C for 10 minutes, and slowly cooled to room temperature. The annealed products were electrophoresed through an 8% (w/v) polyacrylamide gel, and the bands corresponding to the linear duplex DNA of the correct size were sliced from the gel. DNA was recovered by electroelution and extracted with isoamyl alcohol to remove ethidium bromide. A non-specific control DNA was composed

of the two 66 bp *hin*FI fragments from bacteriophage ϕX174 (Life Technologies, Inc.). Gel mobility shift experiments were performed as described (Hengen *et al.* (1997) *supra.*).

Paragraph beginning at line 2 of page 55 has been amended as follows:

A single very long nucleic acid (SEQ ID NO:15) is synthesized having a sequence in the center that causes a hairpin loop to form rapidly (*see,* Figure 11). The entire DNA can then be dissolved in a buffer heated and cooled thereby forming double stranded DNA. This guarantees that the complementary strands are equimolar and there isn't any single-stranded DNA present in the mixture.